

Marked-up set of claims for 09/917,384

We claim:

1. A composition comprising a substantially purified thermostable Gux1 peptide, said Gux1 peptide comprising a catalytic domain GH48, a carbohydrate binding domain (CBD) type III, and a carbohydrate binding domain (CBD) type II.
2. The composition of claim 1 wherein the Gux1 peptide is further defined as comprising a linker and a signal peptide.
3. The composition of claim 1 or 2 wherein the GH48 catalytic domain of the Gux1 peptide is further defined as having a length of about 637 to about 643 amino acids.
4. The composition of claim 1, 2, or 3 wherein the carbohydrate binding domain (CBD) type III of the Gux1 peptide is further defined as having a length of about 150 to about 156 amino acids.
5. The composition of claim 1, 2, 3, or 4 wherein the carbohydrate binding domain (CBD) type II of the Gux1 peptide is further defined as having a length of about 95 amino acids to about 105 amino acids in length.
6. The composition of claim 3 wherein the GH48 catalytic domain is further defined as the sequence of SEQ ID NO: 5.
7. The composition of claim 4 wherein the carbohydrate binding domain (CBD) type III is further defined as the sequence of SEQ ID NO: 4.
8. The composition of claim 6 wherein the carbohydrate binding domain (CBD) type II is further defined as the sequence of SEQ ID NO: 7.

9. The composition of claim 1 further defined as comprising a sequence of SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 7.
10. A thermal tolerant Gux1 peptide having a sequence of SEQ ID NO: 1.
11. The Gux1 peptide of claim 10 further defined as having a sequence of SEQ ID NO: 2.
12. (Cancelled) An industrial mixture suitable for degrading cellulose, such mixture comprising the Gux1 polypeptide of claim 1.
13. (Cancelled) The industrial mixture of claim 12 further defined as comprising a detergent.
14. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 5.
15. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 80% sequence identity to the nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 5.
16. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 70% sequence identity to the nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 5.
17. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 7.

18. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 4.
19. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 6.
20. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 1.
21. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% identity to the nucleic acid sequence of SEQ ID NO: 2.
22. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence encoding a heterologous protein in frame with the Gux1 peptide of claim 1.
23. The composition of claim 22 wherein the heterologous protein in frame with the Gux1 peptide of claim 1 is further defined as a peptide tag.
24. The composition of claim 23 wherein the peptide tag is 6-His, thioredoxin, hemagglutinin, GST, or OmpA signal sequence tag.
24. The composition of claim 22 wherein the heterologous protein is a substrate targeting moiety.
25. The composition of claim 13 wherein the nucleotide sequence encoding the Gux1 is operably linked to a transcriptional or translational regulatory sequence.

26. The composition of claim 25, wherein the transcriptional or translational regulatory sequence comprises a transcriptional promoter or enhancer.
27. An isolated polypeptide molecule comprising:
 - a) a sequence of SEQ ID NO: 4;
 - b) a sequence of SEQ ID NO: 5;
 - c) a sequence of SEQ ID NO: 6;
 - d) a sequence of SEQ ID NO: 7;
 - e) a sequence of SEQ ID NO: 1; or
 - f) an amino acid sequence having at least 70% sequence identity with the amino acid sequence of a), b), c), d), or e).
28. The polypeptide molecule of claim 27, having at least 90% sequence identity with the amino acid sequence of a), b), c), d), or e).
29. A fusion protein comprising the polypeptide of claim 27 and a heterologous peptide.
30. The fusion protein of claim 29, wherein the heterologous peptide is a substrate targeting moiety.
31. The fusion protein of claim 29, wherein the heterologous peptide is a peptide tag.
32. The fusion protein of claim 31, wherein the peptide tag is 6-His, thioredoxin, hemagglutinin, GST, or OmpA signal sequence tag.
33. The fusion protein of claim 29, wherein the heterologous peptide is an agent that promotes polypeptide oligomerization.
34. The fusion protein of claim 29, wherein the agent is a leucine zipper.

35. (Cancelled) A cellulase-substrate complex comprising the isolated polypeptide molecule of claim 27 bound to cellulose.
36. (Cancelled) A vector comprising the polynucleotide molecule that encodes a polypeptide of claim 27.
37. (Cancelled) A host cell genetically engineered to express the polypeptide molecule of claim 27.
38. (Cancelled) A host cell genetically engineered to express the polynucleotide molecule of claim 27.
39. (Cancelled) The host cell of claim 37 or 38, wherein the host cell is a plant cell.
40. (Cancelled) The host cell of claim 37 or 38, wherein the host cell is a fungi.
41. (Cancelled) The host cell of claim 37 or 38, wherein the host cell is a bacterial cell.
42. (Cancelled) The host cell of claim 37 or 38, wherein the host cell is a bacterial cell.
43. A composition comprising the polypeptide molecule of claim 27 and a carrier.
44. A composition comprising the polypeptide molecule of claim 28 and a carrier.
45. (Cancelled) An isolated antibody that specifically binds to the polypeptide molecule of claim 27.
46. (Cancelled) The antibody of claim 45, wherein the antibody is a polyclonal antibody.

47. (Cancelled) The antibody of claim 45, wherein the antibody is a monoclonal antibody.
48. (Cancelled) A method for producing Gux1 polypeptide, the method comprising:
incubating a host cell genetically engineered to express the polynucleotide molecule of claim 27.
49. (Cancelled) The method of claim 48, further comprising the step of:
isolating the Gux1 polypeptide from the incubated host cells.
50. (Cancelled) The method of claim 48, wherein the host cell is a plant cell.
51. (Cancelled) The method of claim 48, wherein the host cell is a bacterial cell.
52. (Cancelled) The method of claim 48, wherein the host cell is genetically engineered to express a selectable marker.
53. (Cancelled) The method of claim 48, wherein the host cell further comprises a polynucleotide molecule encoding one or more polypeptide molecules selected from the glycoside hydrolase family of proteins.
54. (Cancelled) The method of claim 53, wherein the glycoside hydrolase is a thermostable glycoside hydrolase.
55. (Cancelled) A set of amplification primers for amplification of a polynucleotide molecule encoding Gux1, comprising:
two or more sequences comprising 9 or more contiguous nucleic acids derived from the polypeptide molecule of claim 27.
56. (Cancelled) A probe for hybridizing to a polynucleotide encoding Gux1, comprising:

a sequence of 9 or more contiguous nucleic acids derived from the polypeptide molecule of claim 27.

57. (Cancelled) An assay method for the detection of a polynucleotide encoding Gux1, comprising:

amplifying a nucleic acid sequence with a set of amplification primers comprising two or more sequences of 9 or more contiguous nucleic acids derived from the polypeptide molecule of claim 27; and

correlating the amplified nucleic acid sequence with detected polypeptide encoding Gux1.

58. (Cancelled) A method for assessing the carbohydrate degradation activity of Gux1 comprising:

analyzing a carbohydrate degradation in the presence of Gux1 and a carbohydrate degradation in the absence of Gux1 on a substrate; and

comparing the carbohydrate degradation in the presence of Gux1 with the carbohydrate degradation in the absence of Gux1.

59. (Cancelled) A method for assessing the carbohydrate degradation activity of Gux1 in the presence of an agent of interest comprising:

analyzing a carbohydrate degradation in the presence of Gux1 and a carbohydrate degradation in the presence of Gux1 and the agent of interest on a substrate exposed; and

comparing the carbohydrate degradation in the Gux1 treated substrate with the carbohydrate degradation in the Gux1 treated substrate in the presence of the agent of interest.

60. (Cancelled) The method of claim 59, wherein an increase in carbohydrate degradation activity in the presence of the agent of interest demonstrates stimulation of Gux1 activity and wherein a decrease in carbohydrate degradation activity demonstrates inhibition of Gux1 activity.

61. (Cancelled) The method of claim 58, wherein the carbohydrate is cellulose.
62. (Cancelled) The method of claim 58 wherein the agent of interest is an antibody.
63. (Cancelled) A method for reducing cellulose in a starting material, the method comprising:
administering to the starting material an effective amount of a polypeptide molecule of claim 27.
64. (Cancelled) The method of claim 63, further comprising administering a second polypeptide molecule selected from the glycoside hydrolase family of proteins.
65. (Cancelled) The method of claim 63, wherein the polypeptide molecule of claim 27 is thermostable.
66. (Cancelled) The method of claim 63, wherein the starting material is agricultural biomass.
67. (Cancelled) The method of claim 63, wherein the starting material is municipal solid waste.
68. (Added) The composition of claim 1 further comprising a carrier.
69. (Added) The composition of claim 1 wherein the substantially purified thermostable Gux I peptide is further defined as comprising a heterologous peptide or protein.
70. (Added) The composition of claim 69 wherein the heterologous peptide or protein comprises an immunoglobulin.
71. (Added) The composition of claim 69 wherein the heterologous peptide comprises a histidine tag.
72. (Added) The composition of claim 69 wherein the heterologous peptide comprises a leucine zipper.

73. (Added) The composition of claim 69 wherein the heterologous peptide comprises a fusion protein.